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FRACTIONATION OF WHEY PROTEINS AT THE ELECTROACTIVATION OF WHEY

253.05. Processes and apparatus in the food industry

Abstract of the doctoral thesis in engineering sciences

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RESEARCH CONCEPTUAL FRAMEWORK

Factors influencing industrial development are technological innovations, economic attractiveness, and environmental safety. In the context of the circular economy, complex, non-residual treatment of waste/secondary products is the main factor that complies with both consumer and government requirements and involves initiating extensive research to rectify certain applied technological actions [1, 2]. Sustainable development involves solving environmental and social problems, which requires industrial corporations to implement emerging and innovative technologies [3-5].

The relevance and importance of the topic approached. The thesis presents the development of techniques and technologies for the non-residual processing of dispersed media, in particular whey during electroactivation, which allows the fractionation of whey proteins and the production of protein mineral concentrates enriched with certain protein fractions, which can then be used to fortify various food products by increasing their nutritional value. The necessity of processing biological residues, including whey, which is considered a residual product, and, after many debates, classified as a secondary dairy product obtained from the primary processing of milk, is based both on the high biological value of its solid content, including protein, and on the reduction of environmental pollution [6]. To date , the non-residual processing of whey remains a worldwide problem, due to the fact that approximately 50% of the global volume produced is discharged into wastewater, causing major ecological damage [7-9]. Electroactivation is a sustainable electrochemical activation method for processing dispersed media, especially by-products (whey, buttermilk, etc.), and as an alternative of the conventional methods, it presents an advantage due to its capacity to transform electrical energy into chemical energy [10], allowing the non-residual processing of whey with the recovery of proteins and minerals in mineral protein concentrates and the simultaneous isomerization of lactose into lactulose [11].

The purpose of the thesis: is to develop a non-residual, ecological process for the fractionation of whey proteins recovered in mineral protein concentrates during the electroactivation of dairy by-products, to establish optimal conditions and aspects of whey protein fractionation, and to develop a diaphragm electrolyzer for the electroactivation of whey.

The specific objectives are: to study the processes and methods for treating and fractionation of whey proteins from secondary dairy products, to justify the selection of the electroactivation method for different types of whey; to establish the optimal processing regimes for the electroactivation of secondary dairy products: the geometric layout of the electrolysers and the technical and technological parameters; the electric current density; voltage; specific energy consumption per unit volume; the flow regime of the working liquid (whey) and secondary liquid;

processing time; pH and redox potential (E, mV) values; temperature; determining the degree of extraction of protein fractions in protein-mineral concentrates (PMC); establishing the aspects of protein complexes formation depending on the properties of whey proteins recovered in PMC, elucidation of their fractionation characteristics at different whey electroactivation regimes, and determination of the biological value of protein-mineral concentrates obtained by whey electroactivation; development of a diaphragm electrolyzer for obtaining PMCs enriched with certain protein fractions.

The research hypothesis focuses on the ability of whey proteins to undergo differentiated fractionation depending on the electroactivation regimes, which would allow the recovery of protein mineral concentrates and the production of of predeterminedly enriched protein products that could be used as dietary supplements or for fortifying the food composition.

The novelty and scientific originality consists in establishing the influence of electroactivation regimes, including the design and geometric parameters of electrolysers, for the development of a slotted electrolyzer that ensures non-residual, environmentally friendly processing of whey proteins at the recovery in protein mineral concentrates, simultaneously with the isomerization of lactose into lactulose.

Synthesis of the research methodology and justification of the chosen research methods. The main method was the electroactivation of whey with different solid and protein content in electrolysers with different geometric shapes and technical parameters, at different processing regimes, which allowed the fractionation of whey proteins and their recovery in PMCs, which were subsequently analysed using various classical physicochemical and biochemical methods of analysis: the degree of extraction of whey proteins from whey in PMCs (Q, %) was determined by the Warburg method using a СФ-56 spectrophotometer; the determination of the content of protein fractions (Q_p, %) recovered from whey in PMCs was performed with the 15% SDS-PAGE electrophoretic method. The identification of protein fractions was performed by gel analysis using Phoretix 1D Advans and GelAnalyser 19.1 software. The biological value of PMCs was demonstrated by analyzing the amino acid content in concentrates using the AAA-339M amino analyzer (Czech Republic), which is based on the ion exchange chromatography method; the physicochemical parameters (pH, E, mV) were registered sing the Knick 766 pH meter.

The theoretical relevance and scientific innovation of the work: consists in argumentation of whey protein fractionation according to classical properties, extracted in PMC, during whey electroactivation and the influence of processing regimes on the degree of recovery and enrichment of concentrates obtained with certain whey proteins, which occurs simultaneously with the electroisomerization of lactose into lactulose. The scientific innovation was solved by

identifying the optimal conditions for fractionating of the whey proteins during whey electroactivation: argumentation of the varied extraction of the solid and protein content from different types of whey in protein concentrates according to their physicochemical and biochemical properties; the influence of factors generated during electroactivation, which initiate changes in the protein content extracted in concentrates; the influence of the constructive and geometric parameters of electrolysers on the electroactivation of dispersed media of biological origin; identification of the mechanisms of protein compound formation and the degree of fractionation of whey proteins in concentrates recovered during whey electroactivation; establishment of optimal parameters for the development of a diaphragm electrolyser.

Theoretical significance: for the first time, aspects of whey protein fractionation during the electroactivation of secondary dairy products were stipulated depending on the type of whey processed, the geometric shapes and technical parameters of the electrolysers elaborated, and the treatment regimes (electric current density and the regime of working and secondary liquid discharge). The thesis was based on research and experience accumulated in national and international projects carried out in the Laboratory of Thermal and Hydrodynamic Processes, Institute of Applied Physics: STCU #6011 ”Electrophysical processing of whey to obtain health products and environmental protection: technology and installation”, (2015-2017); CSSDT/ANCD/MECC 15.817.02.07A ”Charge, heat and mass tranfer under thermoelectrophysical and cavitational influences; tehnological and technical elaborations”, (2015-2019); 20.80009.5007.06 State Program, ”Intensification of transfer and processing processes in electric, electromagnetic, cavitational fields; applicability”, (2020-2023); Subprogram 011203 "Research and development of the advantages of electroconvection, electroactivation, magnetic fluidization in intensifying heat transfer and processing," (2024-2027).

Applicable value of the work: A method for fractionating serum proteins during the electroactivation of whey was developed, and the optimal technical and technological parameters for the electroactivation of different types of whey were established. The differentiated fractionation of serum proteins from whey in PMCs with simultaneous isomerization of lactose into lactulose was demonstrated, and concentrates enriched with certain protein fractions beneficial to health were obtained. The "Slot electrolyzer" with a semi-cylindrical casing and slots has been developed and patented. It provides for a reduction in specific energy consumption per unit volume by eliminating "dead"/inefficient areas and increasing the surface area of activation. Additionally, it enhances the degree of extraction of protein fractions in PMCs, which occurs alongside the simultaneous isomerization of lactose into lactulose. The technological scheme for producing PMC with a predetermined protein content was developed.

Approval of the work at national and international scientific forums. The results obtained during the course of the work were presented and discussed at 17 national and international conferences and invention salons (International Conference "Modern Technologies in the Food Industry", Chisinau (2016, 2018, 2022, 2024); The International Conferences Celebrating 55 Years of Higher Education and 40 Years of Technical Higher Education into "Vasile Alecsandri", Bacau (2016); International Scientific Conference on Microbial Biotechnology, Chișinău (2016); Conference of students, master's and doctoral students, Chișinău (2021, 2022, 2023, 2024); International Conference on Nanotechnologies and Biomedical Engineering (2019, 2021, 2023); International Conference "Agriculture for Life, Life for Agriculture" (2019, 2020, 2023); International Conference Intelligent Valorisation of Agro-Food Industrial Wastes (2021); National Scientific Conference with International Participation "Integration through Research and Innovation" (2024); International Conference on Materials Science and Condensed Matter Physics (2016, 2018, 2024); 9th International Conference "Microelectronics and Computer Science" & The 6th Conference of Physicists of Moldova, Chișinău (2017) and invention fairs (International Invention Fair INVENTCOR (2021, 2022); International Exhibition of Inventions INVENTICA, Iasi, Romania (2020, 2022); International Exhibition of Inventions and Innovations "Traian Vuia", Timișoara, Romania (2020, 2022); European Exhibition of Creativity and Innovation EUROIVENT (2021, 2022); INFOINVENT International Specialized Exhibition (2019, 2021); International Fair of Innovation and Creative Education for Youth ICE-USV, Suceava, Romania (2023); International Salon of Inventions and Innovative Entrepreneurship, Chisinau (2024).

Publications related to the thesis topic. The research results and issues addressed in the thesis have been published in **52** scientific papers, including **18** scientific articles, **3** invention patents, **9** articles in collections, and **22** abstracts at national and international scientific events.

Summary of the thesis chapters. The paper is presented on **117** typed pages and includes the following chapters: annotation in Romanian, Russian, and English, introduction, **4** chapters, conclusions and recommendations, bibliography with **343** sources, and **5** annexes. The thesis is illustrated with **20** tables and **114** figures.

Keywords: electroactivation, electrofractionation, electrolysers, secondary dairy products, whey, whey proteins, protein fractions.

THE CONTENT OF THE THESIS

1. Electroactivation of whey – sustainable method, conception, processing, utilization, and benefits

Chapter 1 represents a synthesis of information from the specialized literature regarding the composition and physicochemical and biological properties of whey as a secondary dairy product. In detail, data revealing the particularities of whey production, classification, and chemical composition are presented. It provides detailed data on the specifics of whey production, classification, and chemical composition. Special attention is dedicated to the protein content of whey, namely the whey proteins of this liquid, which have the highest nutritional and biological value of all dairy products. In particular, a structure description is provided, with reference to their biological properties, analyzing the aspects of aggregation and the action of physicochemical factors (pH, redox potential, temperature) on the aggregation process. The mechanisms of isomerization of lactose into lactulose are described, in particular LA-transformation and Amadori rearrangement, which allow to understand various aspects of fractionation of whey proteins under different processing conditions according to their properties. The methods of whey processing are analyzed, which contribute to the valorisationn of all their components. The characteristics of the main whey protein products and their functional and nutritional properties and health benefits are presented.

2. Materials and methods

Chapter 2 describes: the materials used for research (whey, secondary liquid, reagents, and consumables); experimental layout of diaphragm electrolysers; methodology for obtaining mineral protein concentrates; physicochemical and biochemical methods used in analytical determinations; methods for identifying protein fractions; statistical processing of experimental data.

Three types of whey with different protein contents (supplied by the joint-stock company "JLC", Chișinău, RM) were electroactivated: whey with high protein content (WHPC) – whey obtained after the production of "Grăuncior" granulated cottage cheese; whey with medium protein content (WMPC) – whey obtained after the production of cottage cheese with 5% fat content; whey with low protein content (WLPC) – whey obtained after the production of the ”Curd product” with 18% fat content.

Various diaphragm electrolysers, conventionally named EDP-2, EDP-4, EDP-5, EDC-3, and EDC-pilot, with certain geometric parameters and technical specifications that allows for non-residual processing of whey, destined for periodic and continuous treatment of whey (working liquid/cathodic liquid (WL)) and secondary liquid (anodic liquid (AL)). The electrolysers were developed at the Laboratory of Thermal and Hydrodynamic Processes at the Institute of Applied

Physics, MSU. The EDP-2, EDP-4 and EDP-5 electrolysers (Fig. 2.2 A, 2.3) were developed in the form of a parallelepiped, and the EDC-3 and EDC-pilot electrolysers (Fig. 2.2 B) contain a semi-cylindrical dielectric casing with anode and cathode cells and a membrane located on the semi-cylindrical casing (Tab. 2.1).

Table 2.1. Main constructive parameters of electrolysers

	EDP-2	EDP-4	EDP-5	EDC-3	EDC-pilot
Distance C-A, l_1 (mm)	18,0	18,0	5,0	30,0	30,0
Distance C-M, l_2 (mm)	10,0	10,0	2,5	15,0	10,0
Distance M-A, l_3 (mm)	8,0	8,0	2,5	15,0	10,0
V/S, mL/cm^2	1,4	1,0	0,3	2,0	0,75
l_1 – the distance between the cathode (C) and the anode (A); l_2 – the distance between the cathode (C) and the membrane (M); l_3 –the distance between the membrane (M) and the anode (A)					

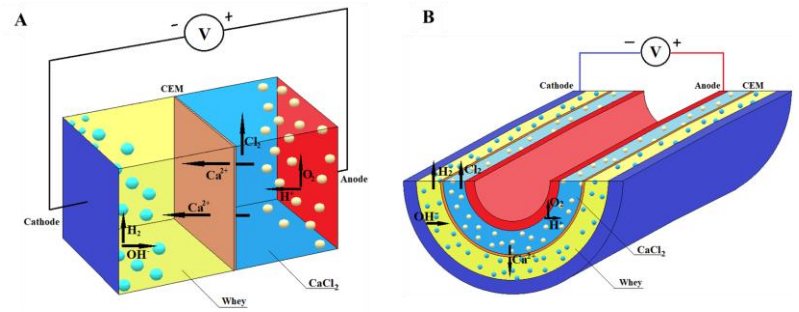


Fig. 2.2. Layout of diaphragm electrolysers: A – EDP-2 and EDP-4; B – EDC-3 and EDC-pilot

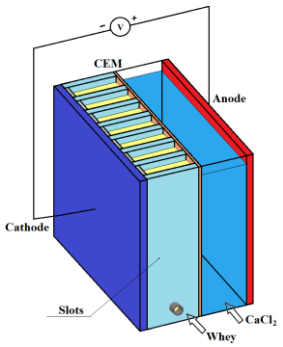


Fig. 2.3. Layout of the EDP-5 diaphragm electrolyzer with slots

3. Establishment of the optimal regimes for the electroactivation of secondary dairy products with the recovery of protein fractions in the protein mineral concentrates

In Chapter 3, the following was investigated: periodic and continuous electroactivation of whey with the establishment of the main optimal parameters depending on the whey processed in electrolyzers developed with different geometric shapes (with a parallelepiped or semi-cylindrical casing) and technical and technological parameters (distance between electrodes, type of used membrane, solid content of initial whey, volume of processed whey (i.e., V/S ratio, mL/cm^2), concentration of calcium ions $v(Ca^{2+})$, mol in secondary (anodic) liquid). The particularities of PMC extraction were determined depending on the characteristics of the parameters: electrical (voltage U,V; specific energy consumption per unit volume A_{SV}, W·h/mL), thermal, physicochemical (pH, redox potential E, mV), biochemical (the degree of extraction of whey proteins in PMC, Q,%) during periodic and continuous electroactivation of different types of whey in different electrolyzers.

3.1. Electroactivation of whey in periodic regime

The processing in a periodic flow regime of whey and anodic liquid aims to highlight the multiple reactions that occur during the electroactivation of all the components in the solid content of the whey. These reactions are influenced both by external factors (such as electrical parameters) and by internal interactions generated by the action of the electric field. These interactions manifest differently when treating different types of whey in various electrolyzers.

3.1.1. Characteristics of electrical parameters during electroactivation in periodic regime

The main electrical parameters during the electroactivation of whey are the electric current density, which is kept constant throughout the processing at j = 10 and 20 mA/cm². However, the voltage (U, V) varies, indicating the conductivity of the processed medium.

The variation in voltage, energy consumption, and specific energy consumption per unit volume (A_{SV}, W·h/mL) at the electroactivation of three types of whey, WHPC, WMPC, and WLPC, in a periodic flow regime of whey and anodic liquid (AL) at electric current densities of 10 and 20 mA/cm² in different electrolyzers depends on several factors: the volume of processed whey, the constructive/geometrical parameters (such as a parallelepiped or semi-cylindrical casing), the solid content of the initial whey, and the concentration of calcium ions in the secondary liquid. The total specific energy consumption per unit volume during the electroactivation of whey, in periodic treatment regime at the electric current densities of 10 and 20 mA/cm² in different electrolysers, indicates the reasonability of using of the electrolyzer EDC-3 (Figs. 3.1-3.3).

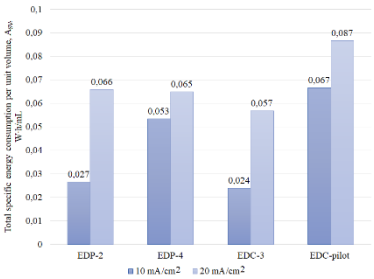


Fig. 3.1. Total specific energy consumption per unit volume, A_{SV}, W·h/mL, at the electroactivation of WHPC in different electrolyzers at j= 10 and 20 mA/cm²

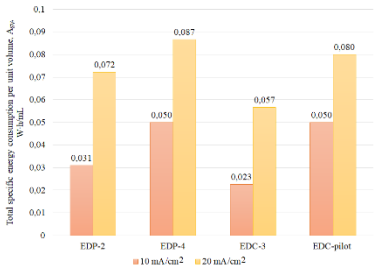


Fig. 3.3. Total specific energy consumption per unit volume, A_{SV}, W·h/mL, at the electroactivation of WMPC in different electrolyzers at j= 10 and 20 mA/cm²

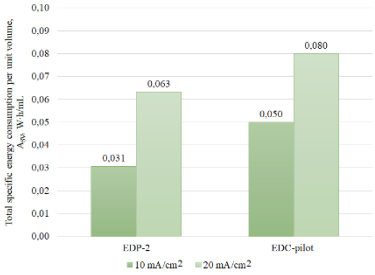


Fig. 3.4. Total specific energy consumption per unit volume, A_{SV}, W·h/mL, at the electroactivation of WLPC in different electrolyzers at j= 10 and 20 mA/cm²

3.1.2. Characteristics of thermal parameters at the electroactivation of different types of whey in various electrolyzers in periodic regime

Whey proteins have a denaturation temperature value of 55-60°C, and their extraction into protein concentrates requires certain processing conditions to be met. Electroactivation of different types of whey in different electrolyzers requires correct management of processing regimes, which is caused by Joule heating, dependent on the conductivity of the treated medium, and generates the formation of two phases: the foamy and liquid phases, which allowed registration of the

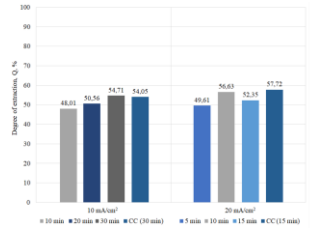
temperature in the liquid phase (t_L,°C) and the foamy phase (t_F,°C). The electroactivation of different types of whey in the electrolyzers with different constructive/geometric parameters and at different processing regimes, does not allow the thermal denaturation of whey proteins during their recovery in PMCs, thus, the process makes it possible to obtain high-quality protein products.

3.1.3. Characteristics of physicochemical parameters at the electroactivation of different types of whey in various electrolyzers in periodic regime

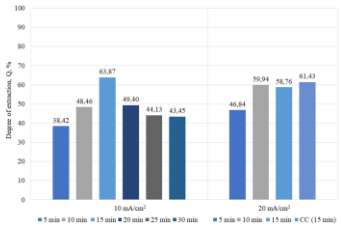
The values of the main physicochemical parameters – pH and redox potential E, (mV) – during the electroactivation of the different types of whey in different electrolysers with different constructive/geometric parameters, vary due to the characteristic reactions of water dissociation in the cathode and anode cell at the electrode surfaces. These variations characterize the physicochemical and biochemical changes occurring in the whey. The variation in pH values and redox potential demonstrates the influence of solid and protein content on the electroactivation of different types of whey in electrolysers with different geometric shapes and technical parameters, caused by the transition of aquacomplexes into hydroxocomplexes, generating a series of inter- and intramolecular changes through the activation of all substances in whey.

3.1.4. Characteristics of of biochemical parameters at the electroactivation of different types of whey in various electrolyzers in periodic regime

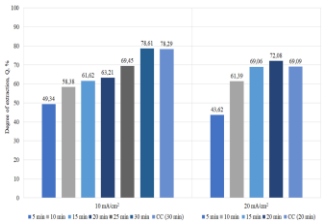
The maximum degree of extraction (Q, %) of whey proteins in PMC was registered at the electroactivation of WHPC (78,61% – foamy phase and 78,29 – liquid phase) and WMPC (75,92% – foamy phase and 78,29% – liquid phase) at the electric current density of j=10mA/cm^2, in the electrolyzer EDC-3 with a semi-cylindrical casing (Figs. 3.4, 3.5).



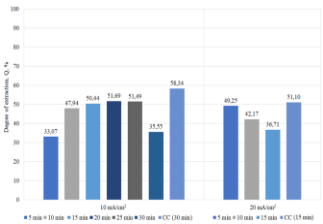
A – EDP – 2



B – EDP – 4

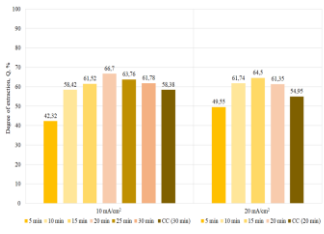


C – EDC – 3

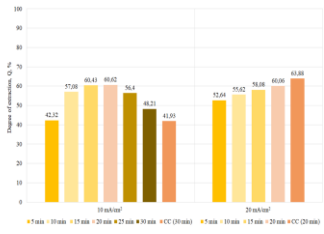


D – EDC - pilot

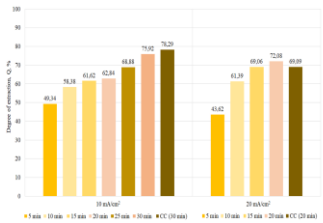
Fig. 3.4. Degree of extraction (Q, %) of whey proteins at the at the electroactivation of WHPC in different electrolyzers at j=10 and 20mA/cm^2: A – EDP – 2; B – EDP – 4; C – EDC – 3; D – EDC - pilot



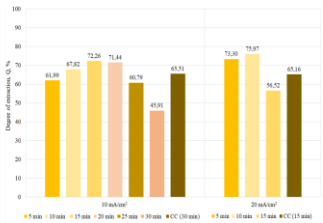
A – EDP – 2



B – EDP – 4



C – EDC – 3



D – EDC - pilot

Fig. 3.5. Degree of extraction (Q, %) of whey proteins at the at the electroactivation of WMPC in different electrolyzers at j=10 and 20mA/cm^2: A – EDP – 2; B – EDP – 4; C – EDC – 3; D – EDC - pilot

The recovery of whey proteins in PMC during the electroactivation of different types of whey is different, uneven, and conditioned, first of all, by the properties of each fraction and their behaviour during electrochemical activation, by the initial solid content, especially the protein content, the constructive/geometric parameters, the processing regimes, the electrical, thermal, and physicochemical parameters. These factors highlight the need for an individual approach to each type of whey, based on its initial solid content, which requires the specification of appropriate technical and technological treatment conditions.

3.2. Electroactivation in continuous regime

Electroactivation of whey in continuous regime was intended to improve and adjust the processing of whey at industrial scale based on research carried out in the periodic flow regime. The electroactivation, in continuous flow regime, of varios types whey in the cathode cell was investigated in three electrolysers: EDC-3, EDP-2 and EDP-5. The electrolyzer EDP-5 was created and developed with slots. The adaptation of the slots has two goals: one technical—to fix the heterogeneous cationic membrane MK-40—and one technological—to increase the surface of activation, thereby, to increase the degree of recovery of protein fractions in PMCs and, respectively, to reduce the specific energy consumption per unit of volume. The electric current density j= 20mA/cm^2, was maintained constant throughout the processing.

3.2.1. Characteristics of electrical parameters at the electroactivation in continuous regime

The total specific energy consumption per unit volume A_{SV} (W·h/mL) during electroactivation in continuous regime of WMPC in different electrolysers (EDP-2, EDC-3, and EDP-5) at j= 20mA/cm² determines the energy efficiency of using slots in the EDP-5. However, there are are limitations to the processing capacity of this electrolyzer (slots size, geometric configuration, the flow of whey in CC, the concentration of calcium ions in AC) (Fig. 3.6).

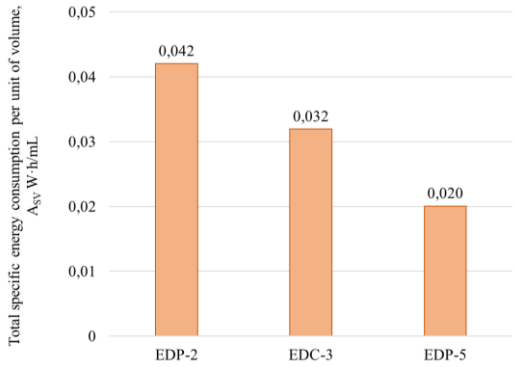


Fig. 3.6. Total specific energy consumption per unit volume A_{SV} (W·h/mL) at the electroactivation of WMPC, continuous regime, j=20mA/cm^2, in different electrolyzers

3.2.2. Characteristics of thermal parameters at the electroactivation of whey in continuous regime

The variation of temperature t,°C during electroactivation of WMPC, in continuous regime of treatment at j= 20mA/cm^2 in different electrolysers, depends significantly on the specific energy consumption per unit volume, the global ohmic resistance and the whey flow rate per unit of initial volume, all of which causes to Joule heating. The electroactivation of WMPC in EDC-3 shows a lower temperature increase in the liquid phase, due to the reduction of the global ohmic resistance and the higher processing capacity compared to EDP-2 (parallelepiped-shaped casing), and excludes the thermal denaturation of whey proteins extracted in PMCs. From a technical point of view, the development of an electrolyzer with slots and a semi-cylindrical casing is reasonable in the context of maintaining a low processing temperature and excluding the Joule heating effect.

3.2.3. Characteristics of physicochemical parameters at the electroactivation of whey in continuous regime

The variation of physicochemical parameters (pH, and E, mV) during electroactivation of WMPC, in continuous regime at an electric current density of 20mA/cm^2 in different electrolyzers, indicates the transition of aquacomplexes into hydroxo-complexes in the electrolyzer EDC-3, which has the highest processing capacity, compared to EDP-2 and EDP-5, where this transition was practically impossible to identify (samples were collected every 5 min), probably due to the rapid transition of aquacomplexes into hydroxo-complexes.

3.2.4. Characteristics of biochemical parameters at the electroactivation of whey in continuous regime

The electroactivation of WMPC during continuous flow regime of whey and anodic liquid in electrolysers with different processing capacities (EDP-2, EDC-3, EDP-5), allowed for the uneven extraction of whey proteins in PMCs. The recovery of whey proteins in PMCs during electroactivation of WMPC in the continuous regime at j=20mA/cm^2 shows a maximum recovery of approximately 70-74% at the treatment in the electrolyzer EDC-3 with a semi-cylindrical casing (Fig. 3.7-3.9).

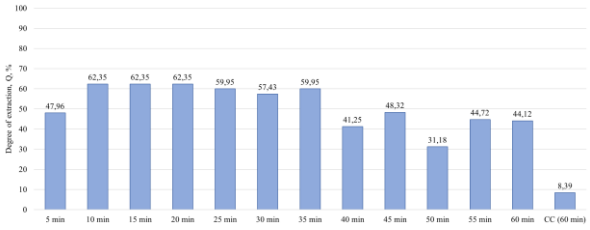


Fig. 3.7. Degree of extraction Q, % of whey proteins in PMC at the electroactivation of WMPC in continuous regime, in EDP-2, at j=20mA/cm^2

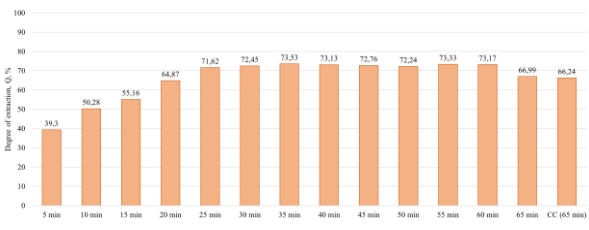


Fig. 3.8 Degree of extraction Q, % of whey proteins in PMC at the electroactivation of WMPC in continuous regime, in EDC-3, at j=20mA/cm^2

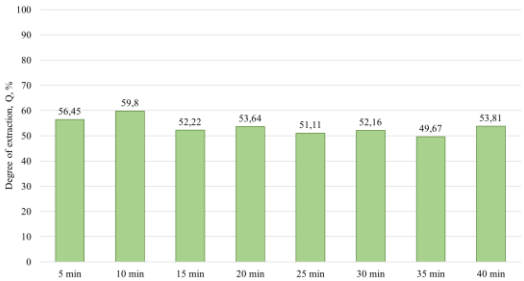


Fig. 3.9. Degree of extraction Q, % of whey proteins in PMC at the electroactivation of WMPC in continuous regime, in EDP-5, at j=20mA/cm^2

4. Electrofractionation of whey proteins and the aspects of protein compounds formation

In Chapter 4, the following results are described and analysed: fractionation of whey proteins during periodic and continuous electroactivation of whey in both periodic and continuous regimes in different electrolyzers, developed with various geometric shapes and technical and technological parameters; aspects of protein compounds formation as a result of whey electroactivation; determination of the biological value of PMCs obtained during whey electroactivation. A separate section is dedicated to the development of the slotted electrolyzer and the technological scheme of the process for obtaining mineral protein concentrate and deproteinized whey, which contains isomerized lactulose from lactose.

4.1. Fractionation of whey proteins at electroactivation

The electroactivation of whey with the electrolyzers studied, at different processing regimes (periodic and continuous, j=10 and 20mA/cm^2), allowed the identification of several protein fractions, which were separated into four groups: high molecular weight proteins (HWP), in which were identified 2-5 fractions with molecular weight (MW) variations of 54-249 kDa, including bovine serum albumin (BSA) with MW 66 kDa, lactoperoxidase (LP) with MW 78 kDa and lactoferrin (LF) with MW 80 kDa, as well as protein complexes with high MW of about 200-249 kDa; caseins (CSN), in which 2-3 fractions were identified—α-CSN, β CSN, and κ-CSN with respective MWs of 37, 33, and 46 kDa; β-lactoglobulin (β-Lg) with MW 18,2; α-lactalbumin (α-La) with MW 14,2 kDa.

4.1.1. Electrofractionation of whey proteins at the electroactivation of whey in periodic regime

Fractionation of whey proteins at the electroactivation of whey in the EDP-2 electrolyzer. Electroactivation of different types of whey in EDP-2, V/S ratio – 1,4 mL/cm^2, in periodic regime, denotes maximum recovery of β-Lg in PMC in the first minutes of processing of the WMPC (approximately 70%) at j=10mA/cm^2 and maximum recovery of α-La in PMC (approximately 70%) during the electroactivation of the WLPC, j=20mA/cm^2, towards the end of the processing (after 20 min of treatment) (Fig. 4.1, 4.2).

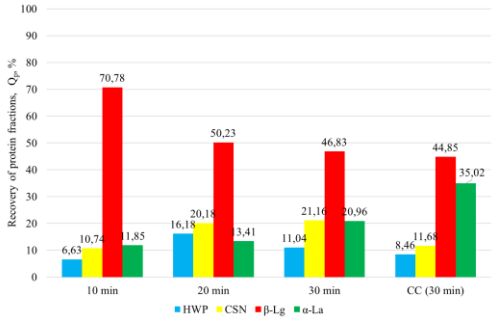


Fig. 4.1. Recovery Qp, % of protein fractions in PMCs, obtained by electroactivation of WMPC in EDP-2, periodic regime, j=10mA/cm^2

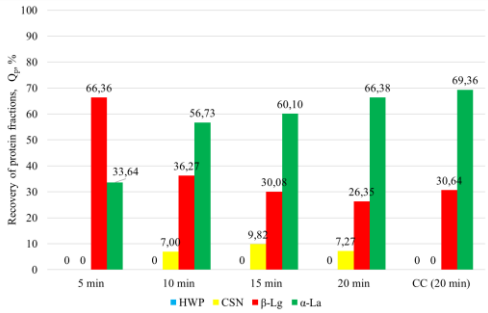


Fig. 4.2. Recovery Qp, % of protein fractions in PMCs, obtained by electroactivation of WLPC in EDP-2, periodic regime, j=20mA/cm^2

The maximal recovery of α-La in PMC (approximately 70%) at the end of processing (after 20 min of treatment) has two explanations: first, the high affinity of α-La for Ca^{2+} ions and other bivalent cations, facilitates the formation of calcium-enriched PMCs; second, α-La is a regulatory protein of the *lactose synthase* enzyme complex, and the lactose concentration in milk is directly related to the α-La content, which hypothetically explains the recovery of this protein into PMC towards the end of processing at the moment of maximal isomerization of lactose into lactulose by the Amadori rearrangement mechanism and the creation of favorable conditions for α-La ”capture” in PMCs. To support this supposition, using the polarimetric method, which allows the determination of the anomeric composition of lactose, it was established that the angle of polarization - α° decreases during electroactivation, which indicates the isomerization of lactose into lactulose, reaching negative values towards the end of processing. For example, the polarization angle α° for the WLPC, upon electroactivation in EDP-2, periodic regime, at j=10mA/cm^2 is (-0,08) at 30 min of treatment and (-0,1) for the CC content, while the α° value of the initial whey is (4,3). Whereas, the polarisation angle α° for the same type of whey at j=20mA/cm^2 is (-1,0) after 20 min of treatment and (-1,4) for the CC content.

Fractionation of whey proteins at the electroactivation of whey in the EDP-4 electrolyzer. The recovery of whey proteins in PMCs during electroactivation in the EDP-4 electrolyzer maintains the electrofractionation character similar to processing in EDP-2. The recovery of whey proteins in PMCs during electroactivation in the EDP-4 diaphragm electrolyzer allows for maximum extraction of β-Lg in the first 5 minutes of processing (43,95%–58,79%), followed by a quantitative decrease towards the end of treatment. α-La extraction increases during processing (14,94%–27%). CSN maintains its recovery within the range of 18.30%–26.17% throughout processing, while HWP extraction is uneven, with maximum values ranging from 19.56% to 29.81%.

Fractionation of whey proteins at the electroactivation of whey in the EDC-3 electrolyzer. The EDC-3 electrolyzer with a diaphragm and semi-cylindrical casing was developed to increase the activation surface area and reduce specific energy consumption per unit volume. It has a V/S ratio of 2,0 mL/cm^2, symmetrical cathode and anode cells, equal distances between electrodes and membrane, and a higher processing capacity, which ensures the exclusion of "dead zones"/inefficient areas, compared to electrolyzers with a parallelepiped-shaped casing.

The recovery of β-Lg, as the major protein fraction in whey, is maximal (approximately 73-92%) in the first minutes of processing in all configurations studied, while α-La has an ascending accumulation tendency in PMCs towards the end of treatment (Fig. 4.3).

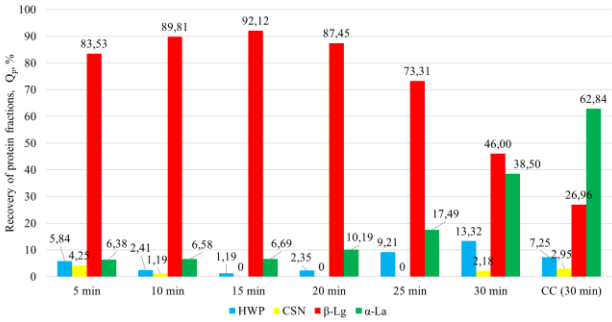


Fig. 4.3. Recovery Q_p, % of protein fractions in PMCs, obtained by electroactivation of WHPC in EDC-3, periodic regime, j=10mA/cm^2

Electroactivation of WHPC in the EDC-3 diaphragm electrolyzer, periodic regime, j=20mA/cm^2, allowed the fractionation of whey proteins and the obtaining of two mineral protein concentrates, conventionally named PMC$_1$ and PMC$_2$, which differ in protein content. CPM$_2$ forms on the surface of the supernatan (deproteinized whey – DW) when separated in a mass field (centrifugation G=1500), while CPM$_1$ sediments. CPM$_2$ is obtained towards the end of electroactivation (15-20 min and in CC content at j=20mA/cm^2), as a result of the "capture" of whey proteins when binding with whey lipids, probably both lipoproteins and low molecular weight proteins (β-Lg, α-La), but also peptides that form as a result of whey proteins hydrolysis at this processing regime (Fig. 4.4, 4.5).

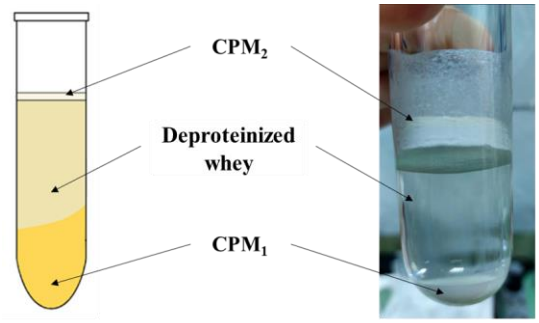


Fig. 4.4. The formation of two mineral protein concentrates, conventionally named CPM$_1$ and CPM$_2$

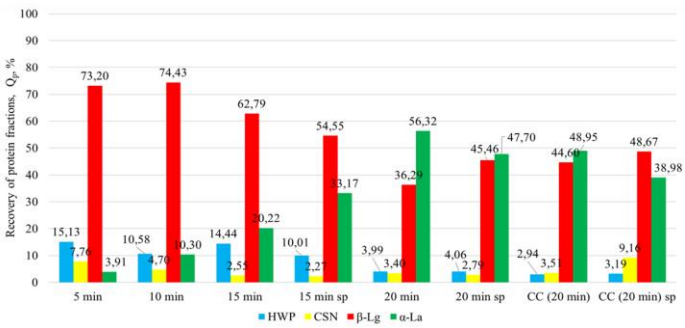


Fig. 4.5. Recovery Q_p, % of protein fractions in PMCs, obtained by electroactivation of WHPC in EDC-3, periodic regime, j=20mA/cm^2

Electroactivation of different types of whey in the EDC-3 semi-cylindrical electrolyzer, in periodic regime, maintains the electrofractionation of major whey proteins and allows the production of two mineral protein concentrates, conventionally named CPM_1 and CPM_2, which differ in protein content, with CPM_2 being obtained towards the end of electroactivation.

Fractionation of whey proteins at the electroactivation of whey in the EDC-pilot electrolyzer. The electroactivation of whey in EDC-pilot, which differs from the other configurations studied, at periodic regime, j=10 and 20mA/cm^2, results in the differentiated recovery of major protein fractions depending on the initial whey used. Processing of the whey with high protein content (HPW) in the EDC-pilot allowed for maximum recovery of β-Lg (approximately 73-76%) in PMCs in the first minutes of treatment, and α-La (approximately 40%, which has an ascending character) towards the end of processing (Fig. 4.6).

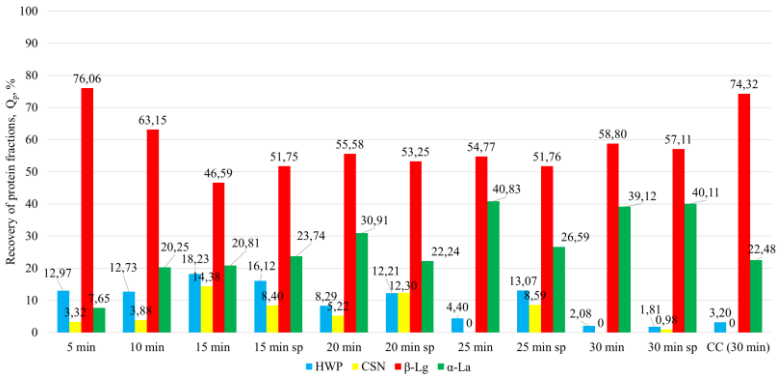


Fig. 4.6. Recovery Q_p, % of protein fractions in PMCs, obtained by electroactivation of WHPC in EDC-pilot, periodic regime, j=10mA/cm^2

The electroactivation of the WMPC and WLPC, in EDC-pilot, periodic regime, j=10mA/cm^2, revealed the intense extraction of α-La in the first minutes of processing (15-20 min) in both the percentage and quantitative analyses (Fig. 4.7, 4.8).

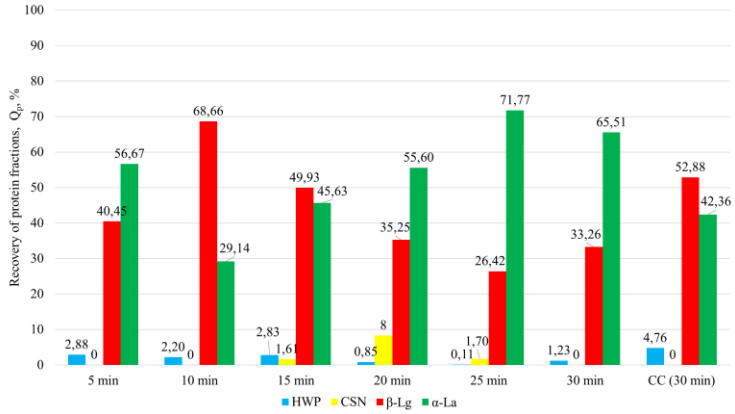


Fig. 4.7. Recovery Q_p, % of protein fractions in PMCs, obtained by electroactivation of WMPC in EDC-pilot, periodic regime, j=10mA/cm^2

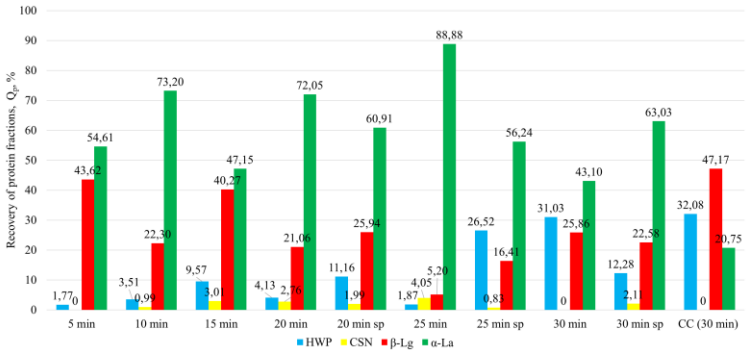


Fig. 4.8. Recovery Q_p, % of protein fractions in PMCs, obtained by electroactivation of WLPC in EDC-pilot, periodic regime, j=10mA/cm^2

A more intense recovery of α-La in PMCs during the first 5 minutes of processing, upon electroactivation of whey in the EDC-pilot diaphragm electrolyzer, led to the investigation of the lactose solution (4%) under the same conditions, to explain the isomerization of lactose to lactulose according to the LA-transformation mechanism and the association of this process with the dynamics of α-La recovery. The study of lactose isomerization into lactulose at the electroactivation of the lactose solution (4%) allowed the identification of a complex formed between isomerized lactulose and calcium ions. The electroactivation of whey, creates favorable condintions for the intense formation of the complex between calcium ions and lactulose by the LA-transformation mechanism. The results highlight the significance of Ca^{2+} ion concentration in relation to the geometric parameters of the electrolysers used, as well as the possibility of adjusting the required Ca^{2+} ion level in the anodic solution. These factors, in turn, influence the isomerisation of lactose into lactulose via both the LA-transformation mechanism and the Amadori rearrangement.

4.1.2. Electrofractionation of whey proteins at the electroactivation of whey in continuous regime.

The fractionation of whey proteins during the electroactivation of whey in the continuous-flow of both the working liquid (whey) and the secondary liquid (anodic liquid) was investigated using two electrolysers: EDP-2 and EDC-3. These electrolysers were selected based on their geometric configuration: EDP-2, with a parallelepiped-shaped casing and a lower specific energy consumption per unit volume compared to EDP-4; and EDC-3, with a semicylindrical casing and a higher processing capacity, yet a lower specific energy consumption per unit volume compared to EDC-pilot, indicating the efficiency of using the EDC-3 electrolyser in continuous processing regime (Fig. 3.2). In continuous regime, the WMPC, which is obtained in large quantities during primary milk processing, was investigated.

Fractionation of whey proteins during electroactivation of whey in continuous regime in the EDP-2 electrolyser. The electroactivation of WMPC in the EDP-2 electrolyser, under continuous regime at j = 20 mA/cm², alowed the maximum recovery of β-Lg in PMCs throughout the entire processing period. α-La, HWP, and CSN are extracted unevenly (Fig. 4.9).

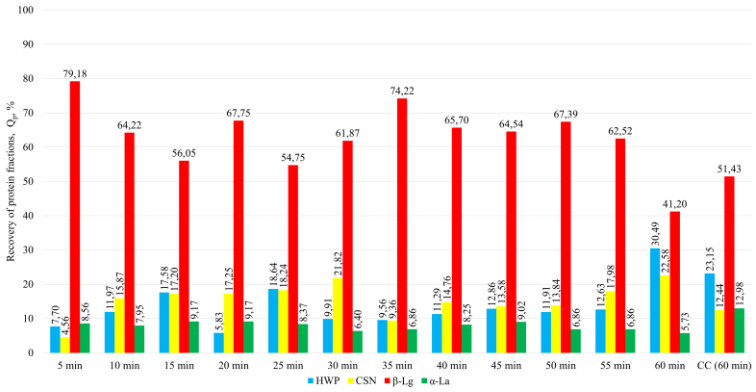
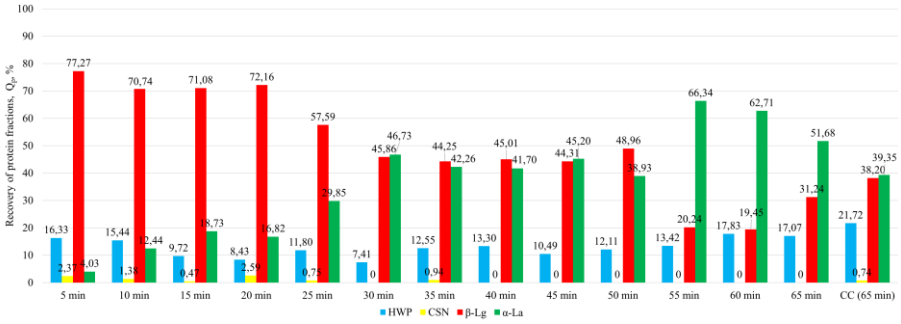


Fig. 4.9. Recovery Q_p, % of protein fractions in PMCs, obtained by electroactivation of WMPC in EDP-2, continous regime, j=20mA/cm^2

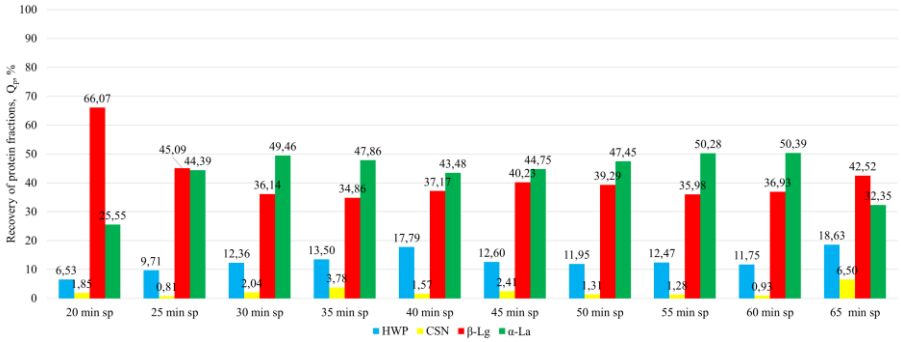
It is worth noting that a higher amount of HWP (30,49%) is recorded in the foam phase after 60 minutes of treatment, most likely due to Archimedean forces involved in ion flotation. In the liquid phase, the content of CC, an accumulation of HWP in PMCs is observed during processing, with a recorded content of 23,15%, and their ”passing“ into the foam phase was not possible as they sediment in the form of aggregates. The uneven recovery is caused both by the geometric shape of the EDP-2 electrolyser and by the WL and AL flow rates. The total specific energy consumption per unit volume is higher compared to the EDC-3, which reduces the energy efficiency of using this type of electrolyser (with a parallelepiped-shaped casing) in continuous processing regime (Fig. 3.6).

Fractionation of whey proteins during electroactivation of whey in continuous regime in the EDC-3 electrolyser. Electroactivation in continuous flow regime of WMPC and anodic liquid in the semicylindrical-casing electrolyzer - EDC-3 at j=20mA/cm^2 allowed the obtaining of two concentrates, CPM_1 and CPM_2, after 20 minutes of processing. The 15% SDS-PAGE analysis of the soluble proteins extracted with pH 8.0 buffer sulution from the concentrates recovered during continuous electroactivation of WMPC shows a high percentage of recovery (Q_p, %) of β-Lg during the first 5 minutes of processing (approximately 77%), which decreases unevenly towards the end of the treatment. The α-La content, on the other hand, is higher towards the end of electroactivation (about 66% at 55 minutes of processing). The same recovery character of the major fractions is maintained in PMC_2, where β-Lg recovery slightly decreases, while α-La recovery increases towards the end of electroactivation, resulting in a differentiated enrichment of

the protein concentrates. HWP recovery is uneven showing an increase (7–21%) towards the end of processing. CSN recovery is minor and non-uniform, with a relatively higher percentage in CPM_2 compared to CPM_1 (Fig. 4.10 A, B).



A – Recovery Q_p, % of protein fractions in PMC_1



B – Recovery Q_p, % of protein fractions in PMC_2

Fig. 4.10. Recovery Q_p, % of protein fractions in PMCs, obtained by electroactivation of WMPC in EDC-3, continous regime, j=20mA/cm^2, A – CPM_1; B – CPM_2

α-La is extracted to a greater extent in CPM_2 compared to CPM_1 starting from the 20 min up to 55 min of processing. Afterwards, its accumulation becomes more intense in CPM_1. A higher recovery of α-La both CPM_1 and CPM_2 is conditioned by the high flux of Ca^{2+} ions, which migrates from the AC and contributes to the ”capture” of α-La, both due to its affinity for Ca^{2+} ions, and to its ”release” from the lactose syntase enzymatic complex during the isomerization of lactose into lactulose, which occurs simultaneously.

The electroactivation of whey in periodic and continuous regimes has made it possible to understand this process from the perspective of researching the recovery of major protein fractions in the PMC, as well as the influence of all electrical, thermal, physicochemical, and biochemical parameters, and the constructive/geometric parameters involved in whey electrofractionation, in order to adapt the process to an industrial scale.

4.2. Aspects of protein compounds formation during whey electroactivation

The electroactivation of whey is accompanied by multiple processes influenced by various factors that determine the formation of protein complexes. The colloidal state of whey is maintained by the whey proteins, which are surrounded by the hidrating membrane.During

electroactivation, this hydrating film is disrupted as a result of the increased salt concentration, due both to the mineral content of each type of whey and to the Ca^{2+} ions that migrate from the anode cell through the heterogeneous MK-40 membrane. This causes protein salinization, which is accompanied by intense foaming of the processed medium. On the other hand, it has been established that during electroactivation in the cathode cell (CC), water dissociation occurs and, as a result, the accumulation and release of hydrogen ions on the cathode surface, contributing predominantly to ionic flotation. This ionic flotation, which occurs simultaneously, in turn intensifies the extraction of PMCs in the form of foam. The initial solid content, especially the protein content of the processed whey type, influences the pH and redox potential values. The increase in pH values during electroactivation indicates the transition of aqua complexes into hydroxo complexes, while the strongly negative redox potential values reveal that the activated whey passes through a series of metastable states haracterized by the presence of hyperactive reductants. After electroactivation, the whey maintains its metastable state during storage, as demonstrated by the variation of pH and redox potential values during relaxation, until thermodynamic equilibrium is reached. Initially, the whey proteins have amphoteric properties (determined by the carboxyl groups –COOH and the amino groups $–NH_2$), whose activation causes oscillatory movements in the CC, leading to various protein aggregations and to the recovery of different fractions under certain electroactivation conditions. The sedimentation of proteins at their isoelectric point (pI) is conditioned by the increase in pH values in CC, both due to the accumulation of hydroxyl groups (OH^-) following the dissociation of water and the activation of the hydrophilic side radicals of amino acids, especially amine groups.

The capacity of the studied electrolyzers, the dimensions of the CC, the flow rates of WL and AL, the periodic or continuous processing regime, and the type of whey electroacivated provide different conditions for reaching the isoelectric point (pI) and, respectively, the sedimentation of certain fractions, which expands during processing.

The formation of high-molecular-weight protein compounds often occurs through the aggregation of proteins with different molecular weights upon activation of sulfhydryl groups (–SH), leading to the formation of disulfide bonds (S–S).Aggregation occurs gradually as the sulfhydryl groups are activated from the exterior of the globule toward its interior, causing the unfolding of the globules. The extraction of caseinates is due to the presence of calcium phosphate–caseinates, where colloidal calcium phosphate forms aggregates with calcium caseinate, creating intermolecular calcium bonds –R–Ca–R–. It is assumed that calcium hydrophosphate or calcium dihydrophosphate ions may also participate in the formation of structural bonds (between two phosphoserine radicals).

4.3. Biological value of protein mineral concentrates obtained at the electroactivation of whey

The biological value of the PMCs obtained at the electroactivation of different types of whey in various electrolyzers is confirmed by the presence of both essential and non-essential amino acids in all the samples studied. The variation of the total, essential, and nonessential free amino acids content upon electroactivation of WHPC and WMPC in the EDP-4 electrolyzer at a current density of j=10-20 mA/cm^2 is shown in Figures 4.11 and 4.12 (A and B).

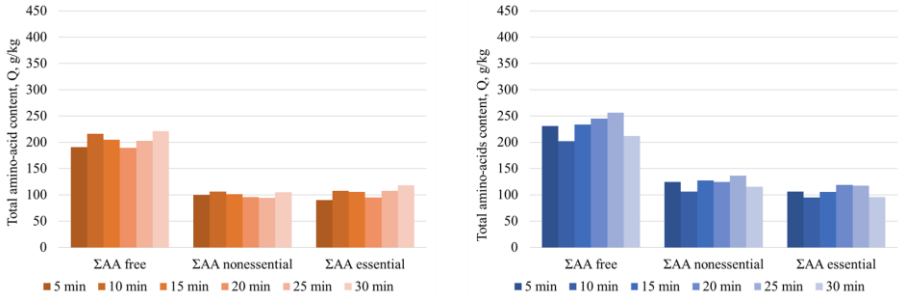


Fig. 4.11. Variation in the total content of free amino acids, non-essential amino acids, and essential amino acids during electroactivation in the EDP-4 electrolyzer of WHPC (A) and WMPC (B) in periodic mode, at a current density of j=10mA/cm^2

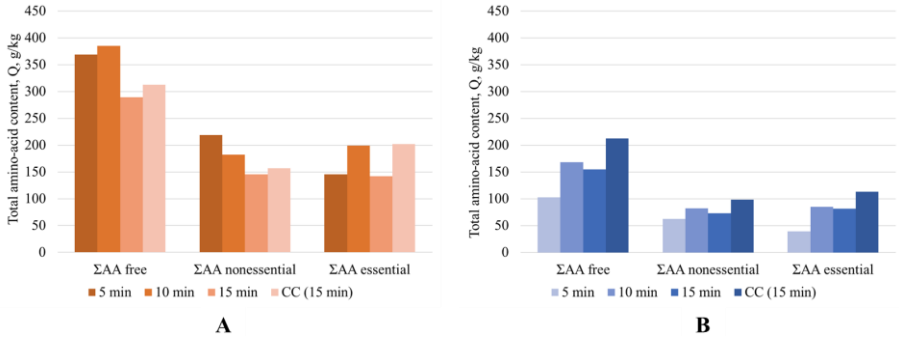


Fig. 4.12. Variation in the total content of free amino acids, non-essential amino acids, and essential amino acids during electroactivation in the EDP-4 electrolyzer of WHPC (A) and WMPC (B) in periodic mode, at a current density of j=20mA/cm^2

Thus, the electroactivation of different types of whey allows the enrichment of PMCs with essential amino acids, whereas the level of migration of both essential and non-essential amino acids into the PMCs varies depending on the processing time and current density. These investigations could be promising in the direction of obtaining PMCs with the desired amino acid content and spectrum by applying different parameters (regimes) of whey electroactivation.

4.4. Development of the slot-electrolyzer

The results of the research on establishing the factors that influence the electroactivation of whey, understanding the physicochemical and biochemical processes that occur when electric current passes through a liquid with a complex biological structure like whey, allowed us to develop the principles and the structural scheme of an Slot Electrolyzer with a semi-cylindrical casing, adapted to the particularities and technological requirements of processing secondary dairy

products for the extraction of protein-mineral concentrates and simultaneous isomerization of lactose into lactulose (Fig. 4.13).

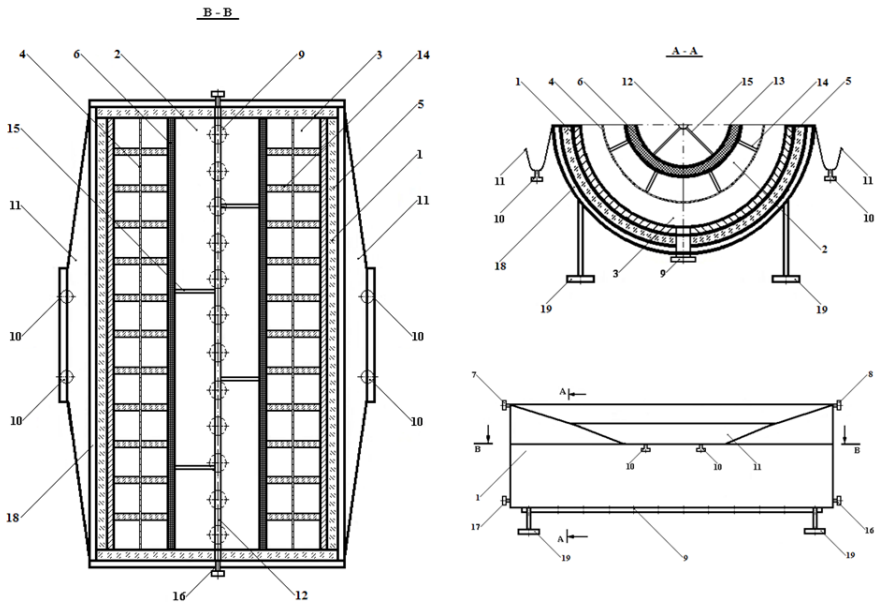


Fig. 4.13. Schematic diagram of the Slot electrolyser:
1 - dielectric body; 2 – anode cell; 3 – cathode cell; 4 - membrane; 5- chatode; 6 - anode; 7 - anodic liquid inlet valve; 8 - anodic liquid outlet valve; 9- initial whey inlet valve; 10 - processed whey outlet valve; 11 - foam collectors; 12 - tubular shaft; 13 - cylinder for fixing the anode; 14 - slots; 15 - radial pipes; 16 - cooling liquid inlet valve; 17 - cooling liquid outlet valve; 18 - cooling jacket; 19 - support brackets.

The slot electrolyzer and a semi-cylindrical casing was developed for the electroactivation of various dispersed media, especially whey, and is intended for continuous processing regime. It increases the activation surface, eliminates 'dead' / inefficient areas, and adapts the working capacity for both laboratory and industrial conditions.

4.5. Technological diagram of the process for obtaining mineral protein concentrate and deproteinized whey

The technological diagram for the process of obtaining protein-mineral concentrates and deproteinized whey, which contains isomerized lactulose, includes the following operations: collecting the whey obtained after cheese or curd production into special containers; cooling the whey to 5-10ºC to stop bacterial activity and prevent the thermal denaturation of whey proteins during electroactivation; electroactivating the whey at an electric current density of 10-20 mA/cm², with a periodic/continuous flow regime of both the working and secondary liquids; separating the deproteinized whey from the protein-mineral concentrates through centrifugation/separation; drying the protein-mineral concentrates by lyophilization; condensing the deproteinized whey through evaporation; and packaging and labeling the final products.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

According to the main objective of the study, the electroactivation of different types of whey in various electrolyzers was investigated, aiming to develop a non-residual, ecological process for fractionating the whey proteins at the recovery into protein-mineral concentrates. This process operates in both periodic and continuous flow regimes of the whey and anodic liquid, and the influence of all the constructive/geometric, electrical, thermal, physicochemical, and biochemical parameters was determined. Thus the following conclusions were drawn:

1. The recovery of whey proteins into PMCs during the electroactivation of different types of whey depends on the geometric configuration, technical and technological parameters, and the capacity of the investigated electrolyzers (V/S ratio, mL/cm²). The maximum degree of protein extraction (Q, %) is observed during the electroactivation of WHPC (78,61% in the foam phase and 78,29% in the liquid phase) and WMPC (75,92% in the foam phase and 78,29% in the liquid phase) using the EDC-3 electrolyzer with a semi-cylindrical casing in a flow regime (j = 10 mA/cm²), and approximately 70–74% during electroactivation in a continuous flow regime of WMPC (j = 20 mA/cm²) (subchapter 3.1.4 and 3.2.4, [324–328]).

2. The specific energy consumption per unit volume, A_{SV}, W·h/mL, during the electroactivation of whey at different processing regimes depends on the processed volume, the solid and protein content of each type of whey, the processing capacity of the electrolyzers, and is attributed to the simultaneous production of two products: protein-mineral concentrate with different whey protein content and deproteinized whey, containing isomerized lactulose. The lowest specific energy consumption is 0,023 W·h/mL at j = 10 mA/cm² and 0,057 W·h/mL at j = 20 mA/cm² for EDC-3 during the treatment of WHPC and WMPC (subchapter 3.1.1., [324-326]). Continuous electroactivation of WMPC in different electrolyzers, EDP-5, EDC-3, and EDP-2 at j = 20mA/cm², determines the energy efficiency of using slot-like structures in EDP-5 (subchapter 3.2.1).

3. The temperature in the liquid phase (t_L,°C) and foamy phase (t_F,°C) during the electroactivation of different types of whey with different electrolysers and constructive/geometric parameters at different processing regimes does not exceed the values of thermal denaturation of whey proteins during their recovery in PMCs and remains within the range of 30-43 °C (subchapter 3.1.2, and 3.2.2 [324-328]).

4. Electroactivation generates the transition of aquacomplexes into hydroxo-complexes, as demonstrated by the modification of physicochemical parameters (variation in pH values and redox potential), which vary depending on the processed whey, the type of electrolyzer used, and the processing regime (subchapter 3.1.3 and 3.2.3, [324-328, 333]). The variations in pH values and redox potential during the storage of electroactivated whey in different electrolyzers and flow regimes (periodic, continuous), are important for explaining the mechanisms of protein comlexes formation during the recovery of whey proteins into PMCs (subchapter 3.1.3 and 3.2.3).

5. The different and uneven recovery of whey proteins in PMCs during the electroactivation of different types of whey in different electrolysers is determined by the properties of each individual protein fraction and their behaviour during electrochemical activation in accordance with the mechanisms of protein complexes formation: ion flotation; salinisation of whey proteins; the transition of aquacomplexes to hydroxo-complexes and the presence of metastable states during storage, which generate multiple inter- and intramolecular transformations; the sedimentation of whey proteins at their isoelectric point; the aggregation of proteins upon activation of sulfhydryl (-SH) groups through the interaction of disulfide (S-S) bonds; formation of calcium phosphate-caseinates (subchapter 4.2, [329, 333, 336]).

6. The differential fractionation of whey proteins during electroactivation varies depending on the processing regimes:

- The recovery of β-Lg, the major protein fraction in whey, is maximal within the first minutes of processing, at approximately 83-92% during the electroactivation of WHPC in EDC-3, j = 10 mA/cm² under periodic regime (subchapter 4.1.1.3, [240, 329]), and about 77% during continuous flow regim of WMPC in EDC-3, j = 20 mA/cm² (subchapter 4.1.2.2).
- α-La is extracted maximally during the electroactivation of WLPC in the EDC-pilot, under periodic regime, j = 10 mA/cm² – about 73,20-88,88% (subchapter 4.1.1.4), and during continuous flow regime of WMPC in EDC-3, j = 20 mA/cm² – about 66% (subchapter 4.1.2.2). The recovery of α-La occurs simultaneously with the maximal isomerization of lactose into lactulose, both through the Amadori rearrangement mechanism and LA-transformation, involving the formation of a complex between Ca^{2+} ions and isomerized lactulose, and the "release" of α-La from the lactose syntase complex, resulting in its recovery into PMCs [48, 239, 330].
- CSN is extracted more intensively – about 29,03%, during the electroactivation of WHPC in EDP-2, under periodic regime, j = 20 mA/cm² (subchapter 4.1.1.1 [336]), and about 15,87-22,58% during continuous flow regime of WMPC in EDP-2, at j = 20 mA/cm² (subchapter 4.1.2.1).
- The HWP content in PMCs is higher during the processing of WHPC in EDP-2, under periodic regime, j = 10 mA/cm² – about 27,16-33,93% (subchapter 4.1.1.1, [29]), and during continuous flow regime of WMPC in EDP-2, at j = 20 mA/cm² – about 18,64-30,49% (subchapter 4.1.2.1).

7. The development of the "Slot Electrolyzer" with a semi-cylindrical casing will allow for a reduction in specific energy consumption per unit volume, an increase in the extraction degree of protein fractions into PMCs, the elimination of 'dead'/inefficient zones, an expansion of the activation surface, and an enhanced rate of lactose isomerization into lactulose."

8. A block diagram has been developed for obtaining protein-mineral concentrates with different protein content and deproteinized whey containing isomerized lactulose.

Electroactivation is a sustainable, ecological, and non-residual method for processing dispersed media, especially secondary dairy products (whey, buttermilk, etc.), which allows for the partial,

and often complete, elimination of chemical reagents, enhances the quality of the final products, reduces specific energy consumption and emissions in solutions, and provides new possibilities for processing.

PRACTICAL RECOMMENDATIONS

The results presented in the thesis allowed for the formulation of practical recommendations regarding the establishment of optimal conditions for whey protein fractionation through the electroactivation of whey and the extraction of major protein fractions in PMCs:

1. The electroactivation of whey in a periodic regime requires maintaining the specific energy consumption per unit volume, A_{SV}, W·h/mL, within the limits of 0,023 W·h/mL at j=10mA/cm² and 0,057 W·h/mL at j=20mA/cm². The electrolyzer capacity is preferentially similar to EDC-3, with V/S=2,0 mL/cm². The specific energy consumption per unit volume is attributed to the production of two final products: PMC and DW, which contains isomerized lactulose derived from lactose.

2. Protein concentrates enriched with β-Lg (with a content of approximately 73-92%) can be obtained at the electroactivation in periodic regime of the WHPC in EDC-3 at j=10mA/cm², and of WMPC in EDC-3 at j=10mA/cm², as well as by the electroactivation in continuous regime of the WMPC in EDC-3 at j=20mA/cm².

3. Protein concentrates enriched with α-La (with a content of approximately 65-88%) can be obtained at the electroactivation in periodic regime of the WLPC in EDC-pilot at j=10mA/cm², and of WMPC in EDC-pilot at j=10mA/cm², while maintaining the calcium ion concentration $\nu(Ca^{2+})$, mol – 0,054 in the anodic liquid. In the continuous regime, α-La enriched concentrates can be obtained by electroactivating WMPC in EDC-3 at j=20mA/cm², maintaining the calcium ion concentration $\nu(Ca^{2+})$, mol – 1,35.

4. Protein concentrates enriched with CSN (with a content of approximately 15-29%) can be obtained at the electroactivation in periodic regime of the WHPC EDP-2 at j=20mA/cm², and at the electroactivation continuous regime of WMPC in EDC-3 at j=20mA/cm².

5. Protein concentrates enriched with HWP (with a content of approximately 18-34%), which include BSA, lactoferrin, lactoperoxidase, and other high molecular weight protein compounds, can be obtained at the electroactivation in periodic regime of the WHPC in EDP-2 at j=10mA/cm², and at the electroactivation continuous regime of WMPC in EDP-2 at j=20mA/cm².

6. It is recommended to use slots similar to EDP-5, according to the invention patent "Slot Electrolyzer", for: reduction in specific energy consumption per unit volume, an increase in the extraction degree of protein fractions into PMCs, the elimination of 'dead'/inefficient zones, an expansion of the activation surface, and an enhanced rate of lactose isomerization into lactulose.

7. The protein mineral concentrates can be used to fortify various nutritional supplements, including those for athletes and infants.

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ADNOTARE

Paladii Irina: ”Fracționarea proteinelor serice la electroactivarea zerului”, teză de doctor în științe inginerești, Chișinău, 2026

Structura tezei: constă din introducere, 4 capitole, concluzii și recomandări, bibliografie cu 343 titluri, 5 anexe. Textul de bază conține 117 pagini, inclusiv 114 figuri și 20 tabele. Rezultatele obținute sunt publicate în 52 lucrări științifice.

Cuvinte-cheie: electroactivare, electrofracționare, electrolizoare, produse lactate secundare, zer, proteine din zer, fracţii proteice.

Scopul lucrării: constă în elaborarea procedeului non-rezidual, ecologic, de fracționare a proteinelor serice recuperate în concentrate proteice minerale la electroactivarea produselor lactate secundare, stabilirea regimurilor optime, aspectelor de fracționare a proteinelor serice și elaborarea electrolizorului cu diafragmă pentru electroactivarea zerului.

Obiectivele specifice sunt: studiul procedeelor şi metodelor de tratare și fracționare a proteinelor serice din produse lactate secundare, argumentarea selectării metodei de electroactivare a diferitor tipuri de zer; stabilirea regimurilor optime de procesare la electroactivarea produselor lactate secundare (forma geometrică a electrolizoarelor și parametrii tehnici și tehnologici; densitatea curentului electric; voltajul; consumul specific de energie pe o unitate de volum; regimul de refulare a lichidului de lucru (zerului) și a lichidului secundar; durata de procesare; valorile pH şi a potențialului redox (E, mV); temperatura; determinarea gradului de extragere a fracțiilor proteice în concentrate proteice minerale (CPM)); stabilirea aspectelor de formare a complecșilor proteici în funcție de proprietățile proteinelor serice din zer, recuperate în CPM, elucidarea particularităților de fracționare a lor la diferite regimuri de electroactivare a zerului și determinarea valorii biologice a concentratelor proteice minerale, obținute la electroactivarea zerului; elaborarea electrolizorului cu diafragmă, destinat obţinerii CPM îmbogățite cu anumite fracții proteice.

Noutatea și originalitatea științifică: constă în stabilirea influenței regimurilor de electroactivare, inclusiv a parametrilor constructivi și geometrici a electrolizoarelor, pentru elaborarea electrolizorului cu fisuri, ce asigură procesarea non-reziduală, ecologică, de fracționare a proteinelor serice ale zerului la recuperarea în concentrate proteice minerale, simultan cu izomerizarea lactozei în lactuloză.

Rezultatele obținute care contribuie la soluționarea problemei științifice importante: a fost elaborat procedeul de fracționare a proteinelor serice la electroactivarea zerului și stabiliți parametrii optimi precum și regimurile de procesare, care permite obținerea concentratelor proteice minerale, îmbogățite cu anumite fracții proteice, simultan cu electroizomerizarea lactozei în lactuloză. A fost demonstrată experimental fracționarea diferențiată a proteinelor serice.

Semnificația teoretică: în premieră au fost stipulate aspectele fracționării proteinelor serice la electroactivarea produselor lactate secundare în dependență de tipul zerului procesat, forma geometrică și parametrii tehnici a electrolizoarelor elaborate, regimurile de tratare (densitatea curentului electric și regimul de refulare a lichidului de lucru și a celui secundar).

Valoarea aplicativă: a fost elaborat: procedeul de fracționare a proteinelor serice la electroactivarea zerului și identificarea parametrilor optimi a regimurilor de procesare; ”Electrolizorul cu fisuri” cu parametri tehnici optimizați; schema-bloc de obținere a concentratului proteic mineral și zerului deproteinizat, ce conține lactuloză izomerizată. Au fost obținute 3 brevete de invenție.

АННОТАЦИЯ

Паладий Ирина: «Фракционирование сывороточных белков при электроактивации молочной сыворотки», диссертация на степени доктора инженерных наук, Кишинев, 2026.

Структура диссертации: состоит из введения, 4 глав, выводов и рекомендаций, библиография в 343 наименованиях, 5 приложения. Основной текст содержит 117 страниц, в том числе 114 рисунков и 20 таблицы. Полученные результаты опубликованы в 52 научных статьях.

Ключевые слова: электроактивация, электрофракционирование, электролизеры, вторичные молочные продукты, молочная сыворотка, сывороточные белки, белковые фракции.

Цель работы: разработка безотходного, экологического процесса фракционирования сывороточных белков, извлеченных в белково-минеральные концентраты (БМК) при электроактивации вторичных молочных продуктов, установление оптимальных режимов, аспектов фракционирования сывороточных белков и разработка диафрагменного электролизера для электроактивации молочной сыворотки (МС).

Задачи работы: изучение процессов и методов обработки и фракционирования сывороточных белков из вторичных молочных продуктов, обоснование выбора процесса электроактивации различных видов МС; определение оптимальных режимов обработки для электроактивации вторичных молочных продуктов: геометрической формы электролизеров и технико-технологических параметров; плотности электрического тока; напряжения; удельного расхода энергии на единицу объема; режима подачи рабочей жидкости (МС) и вторичной жидкости; продолжительности обработки; значения pH и окислительно-востоновительного потенциала (E, мВ); температуры; определения степени извлечения белковых фракций в БМК; выявления аспектов образования белковых комплексов в зависимости от свойств сывороточных белков, извлеченных в БМК и обоснование их фракционирования при различных режимах электроактивации МС; определение биологической ценности, полученых БМК при электроактивации МС; разработка диафрагменного электролизера, предназначенного для получения белково-минеральных концентратов, обогащенных определенными белковыми фракциями.

Научная новизна и оригинальность: заключается в выявлении влияния режимов электроактивации, в том числе конструктивных и геометрических параметров электролизеров, для разработки электролизера с щелями, обеспечивающего безотходную, экологичную переработку молочной сыворотки и фракционирования сывороточных белков при их извлечении в БМК и одновременной изомеризацией лактозы в лактулозу.

Основные результаты: Разработан процесс фракционирования сывороточных белков при электроактивации МС и установлены оптимальные параметры и режимы обработки, позволяющие получать белковые концентраты, обогащенные определенными белковыми фракциями, одновременно с электроизомеризацией лактозы в лактулозу. Экспериментально доказано дифференцированное фракционирование сывороточных белков.

Теоретическая значимость: впервые были представлены аспекты фракционирования сывороточных белков при электроактивации вторичных молочных продуктов в зависимости от вида перерабатываемой МС, геометрической формы и технических параметров разработанных электролизеров, режимов обработки (плотность электрического тока и режимов подачи рабочей и вторичной жидкости).

Прикладное значение: разработаны: способ фракционирования сывороточных белков при электроактивации МС с оптимальными параметрами обработки; «Щелевой электролизер» с оптимизированными техническими параметрами; блок-схема для производства БМК и депротеинизированной сыворотки, содержащая изомеризованную лактулозу. Получено три патента.

ABSTRACT

Paladii Irina: "Fractionation of whey proteins at the electroactivation of whey", PhD thesis in engineering sciences, Chisinau, 2026

Thesis structure: consists of introduction, 4 chapters, conclusions and recommendations, bibliography with 343 titles, 5 annexes. The basic text contains 117 pages including 114 figures and 20 tables. The results are published in 52 scientific papers.

Key words: electroactivation, electrofractionation, electrolyzers, secondary dairy products, whey, whey proteins, protein fractions.

The purpose of the work: consists in the development of the non-residual, ecological process for the fractionation of whey proteins recovered into protein mineral concentrates (PMC) at the electroactivation of secondary dairy products, the establishment of optimal regimes, the mechanisms of serum protein fractionation and the development of the diaphragm electrolyzer for the electroactivation of whey.

The research objectives: the study of the methods and processes of treatment and fractionation of whey proteins from dairy by-products, argumentation of the selected method of electroactivation of different types of whey; determination of the optimal processing regimes for the electroactivation of whey: the geometrical casing of the electrolyzers and their technical and technological parameters; the electric density; voltage; the specific energy consumption per unit volume; the flow regime of the working liquid (whey) and secondary liquid; processing time; pH and redox-potential (E, mV) values; temperature; determination of the recovery degree of whey proteins into PMCs; identification of the mechanisms of formation of protein compounds depending on the properties of serum proteins recovered in PMC and argumentation of their fractionation at different regimes of whey electroactivation; determination of the biological value of protein mineral concentrates; development of the diaphragm electrolyzer designed to obtain PMCs enriched with certain protein fractions.

The scientific novelty and originality: aims to to establish the influence of electroactivation regimes, including the design and geometric parameters of electrolysers, for the development of a slotted electrolyser that ensures non-residual, ecological fractionation of whey proteins during the recovery of mineral protein concentrates, simultaneously with the isomerisation of lactose to lactulose.

Main results: A process for fractionating whey proteins during the electroactivation of whey was developed, and the optimal parameters and processing conditions were established, allowing the production of mineral protein concentrates enriched with certain protein fractions, simultaneously with the electroisomerization of lactose into lactulose. The differentiated fractionation of whey proteins was demonstrated experimentally.

Theoretical significance: for the first time, it was demonstrated the fractionation of whey proteins at the electroactivation of secondary dairy products depending on the type of whey processed, the geometrical casing and technical parameters of the electrolyzer developed, the treatment regimes (current density and the flow regime of the working and secondary liquids).

Applicative value: it was elaborated: the process for the fractionation of whey proteins at the electroactivation of whey and identification of the optimal parameters of the processing regimes; the “Slot electrolyser” with optimized technical parameters; the block-scheme for the obtaining of mineral protein concentrate and deproteinized whey containing isomerized lactulose. 3 patents were obtained.

PALADII IRINA

FRACTIONATION OF WHEY PROTEINS AT THE ELECTROACTIVATION OF WHEY

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